Title: Standardization of minimum inhibitory concentration for polymyxin B using flow cytometry: from hours to minutes.

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Abstract
Introduction. Gram negative rods are the most important bacteria in nosocomial infections. In recent years there has been an increase in the number of infections caused by carbapenem-resistant Gram negatives, which limits treatment options and increases mortality, particularly if diagnosis and therapeutic adjustment are delayed. Rapid diagnosis and correct empirical combination therapy may reduce mortality rates. Currently, reliable antimicrobial susceptibility test results that generates MIC values require 16-20 hours of incubation. Flow cytometry (FC) potentially meets the demand for reducing turnaround time, but requires development, validation and standardization of algorithms for data interpretation. The objective of the work was to develop and validate MIC determination by flow cytometry for polymyxin B in Gram negative bacilli.
Material and methods. The minimum inhibitory concentration (MIC) determined by broth dilution with visual reading and 18 hours of incubation was considered the gold standard. Aliquots of broth dilution with shorter incubation time (90 min) and addition of cell markers was used, and FC was performed in FACS CANTO-II. The standardization was done in Mueller-Hinton-II broth and the same concentrations of polymyxin B (PB) were used for both tests. The strains used were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* 3313 MCR-1 producing, *Klebsiella pneumoniae* J9243971 and *Salmonella sp*. 4370135822, all with MICs simultaneously determined by visual reading.
Results and conclusions. The interpretation criteria for FC results was determined by comparing the absolute number of bacteria at each concentration with the growth control, the pattern of dispersion in two dimensional histograms (cell markers) and the MIC obtained by visual reading. The test was repeated 23 times for *E. coli* ATCC 25922 and only one replicate was done for the other strains. The results indicate an excellent correlation with 100% agreement between the results for FC and visual reading in all 27 tests, using different bacterial genera with varied results: *Escherichia coli* 25922 (0.25–1.0 mg/L), *P. aeruginosa* 27853 (0.5 mg/L), *E. coli* 3313 (4.0 mg/L), *K. pneumoniae* J9243971(0.125 mg/L) and *Salmonella* sp. 4370135822 (0.5 mg/L).

Keywords: Flow cytometry. Microbial drug resistance. Microbial sensitivity test. Fast methods.

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